2004

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J. Med. Entomol. 41(2): 193–200 (2004)

ABSTRACT Amitraz, a formamidine acaricide, plays an important role in the control of the southern cattle tick, *Boophilus microplus* (Canestrini), and other tick species that infest cattle, dogs, and wild animals. Although resistance to amitraz in *B. microplus* was previously reported in several countries, the actual measurement of the level of amitraz resistance in ticks has been difficult to determine due to the lack of a proper bioassay technique. We conducted a survey, by using a newly reported technique that was a modification of the standard Food and Agriculture Organization larval packet test, to measure the levels of resistance to amitraz in 15 strains of *B. microplus* from four major cattle-producing states in Mexico. Low-order resistance (1.68- to 4.58-fold) was detected in 11 of those strains. Our laboratory selection using amitraz on larvae of the Santa Luiza strain, which originated from Brazil, achieved a resistance ratio of 153.93 at F_6, indicating the potential for high resistance to this acaricide in *B. microplus*. Both triphenylphosphate and piperonyl butoxide significantly synergized amitraz toxicity in both resistant and susceptible tick strains. Diethyl maleate synergized amitraz toxicity in one resistant strain but had no effect on the susceptible strain and had minor antagonistic effects on two other resistant strains. Target site insensitivity, instead of metabolic detoxification mechanisms, might be responsible for amitraz resistance observed in the Santa Luiza strain and possibly in other amitraz resistant *B. microplus* ticks from Mexico. The Santa Luiza strain also demonstrated high resistance to pyrethroids and moderate resistance to organophosphates. Multiple resistance shown in this strain and other *B. microplus* strains from Mexico poses a significant challenge to the management of *B. microplus* resistance to acaricides in Mexico.

KEY WORDS amitraz, acaricides, resistance detection, cattle tick, *Boophilus microplus*

AMITRAZ IS A FORMAMIDINE ACARICIDE that has been used effectively in the control of several important agricultural pests, including ticks on cattle (Haigh and Gichang 1980, Davey et al. 1984, Garris and George 1985, Kagaruki 1996), dogs and wild animals (Pound et al. 2000, Elfassy et al. 2001, Kumar et al. 2001), as well as parasitic mites of honey bee (Baxter et al. 1999, Elzen et al. 2001, Floris et al. 2001) and other nonixodidae ectoparasites of livestock (Curtis 1985). It was postulated that formamidine pesticides exert their toxic effect on target pest species by interaction with the octopamine receptor of the central nervous system (Evans and Gee 1980, Dudai et al. 1987), and possibly also by inhibition of monoamine oxidases (Atkinson et al. 1974, Schuntner and Thompson 1976). Although the modes of action for amitraz are not fully understood, amitraz and other formamidines offer a novel class of pesticides with a distinct mode of action. Amitraz has played a critical role in the control of the southern cattle tick, *Boophilus microplus* (Canestrini), in countries where resistance to both organophosphate (OP) and pyrethroid pesticides reached unacceptable levels (Aguirre et al. 1986, Kunz and Kemp 1994, Fragoso et al. 1995, Parrodi et al. 1995).

B. microplus is an important ectoparasite of cattle and the key vector of bovine babesioses in many tropical and subtropical regions of the world (Friedhoff and Smith 1981, Bram et al. 2002). Although it was eradicated from the United States in the 1940s, this pest continues to cause damage in other parts of the world, including Australia, Mexico, and Central and South American countries (Graham and Hourrigan 1977, Fragoso et al. 1995, Kemp et al. 1999, Benavides et al. 2000). Chemical acaricides have played a pivotal role in the control of this economically damaging pest. However, as a consequence of extensive use of chemical acaricides, *B. microplus* developed resistance to major classes of acaricides in several countries. In Mexico, resistance to OP acaricides first developed in the 1980s, and resistance to pyrethroids subsequently developed in the 1990s (Aguirre et al. 1986, Fragoso et al. 1995, Santamaría et al. 1999). Amitraz, along with

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pyrethroids, was introduced to control OP-resistant ticks in Mexico in 1986 (Aguirre et al. 1986, Soberanes et al. 2002). Amitraz use became more prevalent and intensive after pyrethroid resistance was discovered in 1993, and a new formulation of amitraz, a 12.5% emulsifiable concentrate (EC), was adopted for whole-body spray treatment or dipping treatment of cattle infested with OP- or pyrethroid-resistant ticks (Parrodi et al. 1995). The first case of amitraz resistance in *B. microplus* from Mexico was confirmed in the San Alfonso strain collected in 2001 from a ranch near Emiliano Zapata in the state of Tabasco (Soberanes et al. 2002). Resistance to amitraz in *B. microplus* was reported earlier in the Ulam and Ultimo strains in Australia in 1981 and 1992, respectively (Nolan 1981, Kunz and Kemp 1994). In recent years, resistance to amitraz was also found in *B. microplus* populations from Colombia (Benavides et al. 2000), South Africa (Strydom and Peter 1999), and Brazil (Furlong 1999, Miller et al. 2002).

The OP acaricide coumaphos has played a pivotal role in the USDA's Cattle Fever Tick Eradication Program (CFTEP) in preventing *B. microplus* from reentering the United States from Mexico through cattle importation (Graham and Hourrigan 1977, George 1996). Resistance to coumaphos and other OPs found in the Mexican populations of *B. microplus* in recent years prompted efforts to seek an alternative acaricide that could be used in the dipping vats at USDA's cattle import facilities along U.S.–Mexico border. Amitraz is an excellent candidate due to its high efficacy in controlling OP- and pyrethroid-resistant *B. microplus* and its low toxicity to cattle in the dipping vats (Parrodi et al. 1995, George et al. 1998). However, the potential for *B. microplus* from Mexico to develop high level of amitraz resistance is also a major concern to the CFTEP. Knowledge of the distribution and level of amitraz resistance in Mexico, as well as a thorough understanding of the mechanisms that confer resistance in *B. microplus* are crucial to the U.S. CFTEP. We report here the results of a study that used a modified Food and Agriculture Organization (FAO) larval packet test (LPT) (Miller et al. 2002) to measure amitraz resistance in *B. microplus* strains collected from various regions of Mexico and to investigate the mechanisms of resistance with synergist bioassays. We also report the results of a laboratory selection for amitraz resistance and multiple acaricide resistance in a Brazilian amitraz-resistant *B. microplus* strain.

Materials and Methods

Tick Strains. A total of 17 strains of *B. microplus* were evaluated for resistance to amitraz, including 15 from different regions of Mexico, one amitraz-resistant strain from Brazil, and one susceptible strain from Texas. Among the Mexican strains tested, seven (Caporal, San Roman, Linda Vista, Aguada, Zacatal, San Luis, and Duran) were collected from the state of Campeche, four (San Felipe, Guaviota, La Coma, and La Mesa) from the state of Tamaulipas, and two (Coatzacoalcos and Tuxpan) from the state of Veracruz. The other two Mexican strains, Pesqueria and Linares, were collected from cattle originating from the state of Nuevo Leon by USDA Veterinary Service inspectors at the port of entry in Reynosa, Mexico. These Mexican tick strains were established at the USDA CFTRL in Mission, TX, between 1994 and 2001. The Coatzacoalcos and San Felipe strains were challenged with permethrin, and Caporal, San Roman, and Tuxpan strains were challenged with coumaphos to increase and maintain resistance to pyrethroid and OP acaricides, respectively (Davey and George 1998, Miller et al. 1999, Li et al. 2003). The remainder of the Mexican *B. microplus* strains was not exposed to any acaricide during their laboratory colonization.

The Santa Luiza strain, which was resistant to amitraz when colonized (Miller et al. 2002), was originally collected from Brazil, and a colony was maintained at the Centro Nacional de Servicios de Constatacion en Salud Animal, Jiutepec, Morelos, Mexico, before being shipped to the CFTRL in Mission, TX, in 2000. The Gonzalez strain was established from an outbreak of *B. microplus* ticks in Zapata County, Texas, in 1994. It was maintained at the CFTRL without any acaricide exposure since its establishment. The Gonzalez strain was susceptible to major classes of acaricides tested and therefore was used as a susceptible reference strain.

Chemicals. Formulated amitraz (Taktic, 12.5% EC) used in this study is a product of NOR-AM Chemical Company (Wilmington, DE). Technical grade coumaphos with 97.4% active ingredient ([AI]), diazinon (87.6% [AI]), and permethrin (92.2% [AI]) were obtained from BayVet (Shawnee, KS), ECTO Development Corporation (Excelsior Springs, MO), and FMC (Philadelphia, PA), respectively. Three synergists used in this study, triphenylphosphate (TPP, an inhibitor of esterases), piperonyl butoxide (PBO, an inhibitor of oxidases), and diethyl maleate (DEM, an inhibitor of glutathione *S*-transferases), were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Bioassays. All amitraz bioassays were conducted in 2002, except for those of the Pesqueria strain, which were performed in 2001. A top dose of amitraz was prepared by adding a volume of the formulated amitraz to a mixture of trichloroethylene (Sigma-Aldrich, St. Louis, MO) and olive oil (Sigma-Aldrich) diluent with a final 2:1 ratio. Three serial dilutions from the top dose were made using a 2:1 trichloroethylene and oil diluent. Six to nine doses, including the control (diluent only), were prepared for each bioassay, and each dose had three replicates. When a particular synergist was tested with amitraz, the synergist was added into the diluent at a constant rate of 1% before amitraz dilutions were made. A volume of 0.7 ml of each dilution was applied to a piece (7.5 by 8.5 cm) of nylon fabric (type 2320, Cerex Advanced Fabrics, Pensacola, FL). The treated fabrics were placed on a hanging rack in a fume hood for 2 h to allow trichloroethylene to evaporate. The fabrics were then folded in half and sealed with bulldog clips on both sides. Fourteen- to 16-d-old larvae were used in bioassays. A modified FAO larval packet test (Miller et al. 2002) was used to

Table 1. Dose-mortality responses and resistance ratios to amitraz in various strains of *B. microplus* from Mexico

Strain[a]	Origin	Year[b]	*n*	Slope (SE)	χ^2 (df)	LC_{50} (95% CI[c])	RR (95% CI[c])
1. Gonzalez	Texas, US	1994	3,345	3.40 (0.18)	40.7 (18)	0.0070 (0.0062–0.0077)	1
2. Coatzacoalcos	Veracruz	1994	3,004	3.01 (0.18)	89.9 (19)	0.0071 (0.0055–0.0085)	1.01 (0.90–1.14)
3. Tuxpan	Veracruz	1994	2,000	2.60 (0.17)	37.6 (19)	0.0073 (0.0060–0.0086)	1.05 (0.91–1.20)
4. San Felipe	Tamaulipas	1996	2,581	1.50 (0.06)	96.9 (19)	0.0125 (0.0096–0.0160)*	1.79 (1.58–2.04)
5. Linares	Nuevo Leon	2000	2,667	2.91 (0.14)	163.3 (19)	0.0141 (0.0105–0.0177)*	2.03 (1.82–2.26)
6. San Roman	Campeche	1998	2,557	2.43 (0.20)	100.3 (22)	0.0212 (0.0165–0.0260)*	3.05 (2.71–3.43)
7. Pesqueria	Nuevo Leon	2001	2,122	3.00 (0.15)	38.7 (25)	0.0278 (0.0252–0.0304)*	4.00 (3.62–4.42)
8. Caporal	Campeche	1998	2,270	2.40 (0.16)	43.6 (19)	0.0319 (0.0276–0.0368)*	4.58 (4.09–5.12)
9. Aguada	Campeche	2001	2,944	3.37 (0.16)	184.9 (19)	0.0075 (0.0058–0.0091)	1.07 (0.98–1.18)
10. Duran	Campeche	2001	2,406	3.83 (0.27)	132.8 (19)	0.0089 (0.0065–0.0108)	1.28 (1.15–1.42)
11. La Mesa	Tamaulipas	2001	2,214	2.58 (0.18)	57.7 (19)	0.0117 (0.0093–0.0140)*	1.68 (1.48–1.91)
12. Gaviota	Tamaulipas	2001	2,388	3.57 (0.28)	125.7 (19)	0.0137 (0.0095–0.0170)*	1.96 (1.74–2.21)
13. Linda Vista	Campeche	2001	2,645	3.85 (0.23)	108.7 (19)	0.0137 (0.0110–0.0161)*	1.97 (1.73–2.25)
14. San Luis	Campeche	2001	1,959	4.10 (0.25)	37.5 (19)	0.0145 (0.0130–0.0161)*	2.09 (1.89–2.31)
15. Zacatal	Campeche	2001	1640	3.93 (0.26)	42.8 (19)	0.0194 (0.0167–0.0221)*	2.79 (2.50–3.12)
16. La Coma	Tamaulipas	2001	2695	1.84 (0.19)	99.9 (19)	0.0265 (0.0175–0.0354)*	3.80 (–)

[a] The Gonzalez was the susceptible reference strain. Strains 2 and 4 were selected with permethrin, and strains 3 and 5–8 were selected with coumaphos during laboratory colonization. Strains 9–16 were not exposed to any acaricide after their field collection.

[b] Year of collection from field. All bioassays were performed in 2002 except for the Pesqueria strain, to which bioassays were done in 2001.

*, The LC_{50} of the test strain was significantly higher than that of the reference strain.

measure the levels of amitraz resistance in all tick strains, as well as the effects of synergists on toxicity of amitraz in four of the strains. Approximately 100 larvae were placed into each packet with a fine brush, and the top was sealed with another bulldog clip. The packets were then placed in an incubator at 27 ± 2°C, 90% RH for 24 h. Then, the packets were removed from the incubator, and the larval mortality in each packet was determined by counting the live and dead larvae in the packet.

A slightly modified version of the standard FAO larval packet test (Miller et al. 1999) was also used to measure the levels of resistance to coumaphos, diazinon, and permethrin in the Santa Luiza strain compared with the susceptible Gonzales strain.

Selection for Amitraz Resistance. The Santa Luiza strain was challenged with various concentrations of amitraz in 10 of 12 generations after its establishment at the CFTRL. The larvae of first four generations were challenged with 0.2% amitraz, a dose that killed ≈50% of larvae in those generations. F_5 was not challenged due to a schedule conflict. Because older larvae were used for selection at F_6, the challenging dose was reduced to 0.04% amitraz, a dose which would kill 100% of larvae from the susceptible strain. F_7 was again not challenged. The challenging dose for F_8, F_9, and F_{10} were 0.1, 0.2, and 0.5%, respectively, before being reduced back to 0.3% in F_{11} and F_{12}. Amitraz bioassays were performed to monitor the change of resistance to amitraz in most of the generations.

The procedures for rearing ticks on cattle, maintaining the nonparasitic stages in the laboratory and challenging larvae with amitraz were similar to that described by Davey et al. (1980) and Davey and George (1998). The only exception was that amitraz-impregnated nylon fabrics, instead of filter papers, were used for selection of amitraz resistance.

Data Analysis. Probit analysis of dose-mortality data were performed using POLO-PC (LeOra Software 1987). Resistance ratios (RR) were calculated relative to the susceptible Gonzalez strain, and synergism ratios (SR) were calculated relative to the amitraz-only bioassay for the same strain. The RR and SR were generated using the formula described by Robertson and Preisler (1992) that takes into account the variance and covariance of the slope and intercept of both regression lines for comparison at LC_{50}. Difference between LC_{50} estimates was designated as significant if their 95% confidence interval (CI) did not overlap.

Results

Larval Susceptibility to Amitraz. The results of bioassays on larvae of all Mexican strains of *B. microplus* are summarized in Table 1. Strains coded number 1 through 8 have been maintained for various generations at the CFTRL after their collection, and some were subjected to selection with coumaphos or permethrin. The Coatzacoalcos and Tuxpan strains, both collected in 1994, were as susceptible to amitraz as the Gonzalez susceptible reference strain. The amitraz LC_{50} of the San Felipe strain was significantly higher than that of the Gonzalez strain. Resistance ratios between 2 to 3 were detected in the Linares and the San Roman strains. The strains numbered 9 through 16 were relatively new strains collected in 2001 and were not exposed to any acaricide at the CFTRL. The Aguada and Duran strains were as susceptible to amitraz as the Gonzalez reference strain. Significantly higher LC_{50} values were observed in the La Mesa, Gavita, and Linda Vista strains, and resistance ratios in those strains ranged from 1.68 to 1.97. Resistance ratios between 2 and 3.8 were found in San Luis, Zacatal, and La Coma strains.

Selection for Amitraz Resistance in the Santa Luiza Strain. In comparison with the Gonzalez strain the Santa Luiza strain of *B. microplus*, which originated from Brazil, demonstrated a RR of 13.36 at F_1 (Table

Table 2. Increase of resistance to amitraz as a result of selection in the Santa Luiza strain of *B. microplus* originated from Brazil

Strain	Bioassay date	*n*	Slope (SE)	χ^2 (df)	LC_{50} (95% CI[a])	RR (95% CI[a])	Challenge dose[a]
Gonzalez							
F_{32}	2/20/02	3,345	3.40 (0.18)	40.7 (18)	0.0067 (0.0062–0.0077)	1	0
Santa Luiza							
F_1	12/6/00	3,363	2.77 (0.14)	120.3 (34)	0.0930 (0.0795–0.1077)	13.36 (12.03–14.84)	0.2
F_2	2/15/01	3,675	2.75 (0.13)	207.6 (34)	0.1350 (0.1070–0.1651)	19.39 (17.39–21.63)	0.2
F_3	4/23/01	4,437	1.01 (0.04)	816.6 (34)	0.3115 (0.1681–0.6176)	44.76 (38.93–51.46)	0.2
F_6	11/15/01	3,150	9.17 (0.85)	218.5 (25)	1.0713 (0.8554–1.1927)	153.93 (141.84–167.05)	0.04
F_8	4/18/02	3,916	4.82 (0.35)	135.6 (25)	0.4783 (0.4044–0.5438)	68.72 (62.77–75.24)	0.1
F_9	6/12/02	2,258	2.63 (0.19)	193.2 (19)	0.3485 (0.2060–0.4662)	50.07 (44.19–56.73)	0.2
F_{12}	1/13/03	2,109	2.22 (0.12)	182.6 (19)	0.3441 (0.2492–0.4575)	49.43 (44.18–55.30)	0.3

[a] Challenge dose was expressed as amitraz (% AI). F_4 was challenged with 0.2% amitraz; F_5 and F_7 were not challenged; F_{10} and F_{11} were challenged with 0.5 and 0.3% amitraz, respectively.

2). An immediate increase of resistance ratio to 19.39 was observed in F_2 after F_1 larvae were selected with 0.2% amitraz. Continued amitraz selections in the following generations resulted in a sharp increase of LC_{50} estimate. The resistance ratio reached 44.76 and 153.93 in F_3 and F_6, respectively. The relaxation of selection pressure in the F_6 and the absence of challenge in the F_7 generation, respectively, led to a decrease of the resistance ratio in F_8. Although the selection pressure was equal to or above the original amitraz concentration (0.2% [AI]) after F_8, the resistance ratio declined and then stabilized at ≈50 in F_9 and F_{12} (Table 2).

Effects of Synergist on Amitraz Toxicity. The effects of three synergists, TPP, PBO, and DEM, on amitraz toxicity to larvae of the susceptible Gonzalez strain, and those of three amitraz-resistant strains of *B. microplus* are illustrated in Fig. 1. Synergistic effects of TPP and PBO were observed in both susceptible and resistant strains. With a synergism ratio at 2.01, TPP significantly synergized amtraz toxicity in the Gonzalez strain. A similar TPP synergism ratio was also found in the Linares and Pesqueria strains, which had amitraz resistance ratios of 2.03 and 4.00, respectively. The TPP synergism ratio in the Santa Luiza strain was 5.86, which was significantly higher than that of the Gonzalez strain. The Santa Luiza strain demonstrated a RR of 68.72 at the same generation (F_8) when synergist bioassays were conducted. The PBO synergism ratio was 7.56 in the Gonzalez strain. The Pesqueria strain had a PBO synergism ratio at 8.53, which is not significantly different from that of the Gonzalez strain. The PBO synergism ratio in the Santa Luiza strain was 5.85, which was significantly lower than that of the Gonzalez strain. The lowest PBO synergism ratio (2.20) was observed in the Linares strain. DEM had no effect on amitraz toxicity in the Gonzalez strain, whereas a weak antagonistic effect was observed in the Linares and Santa Luiza strains. DEM significantly synergized amtraz toxicity only in the Pesqueria strain.

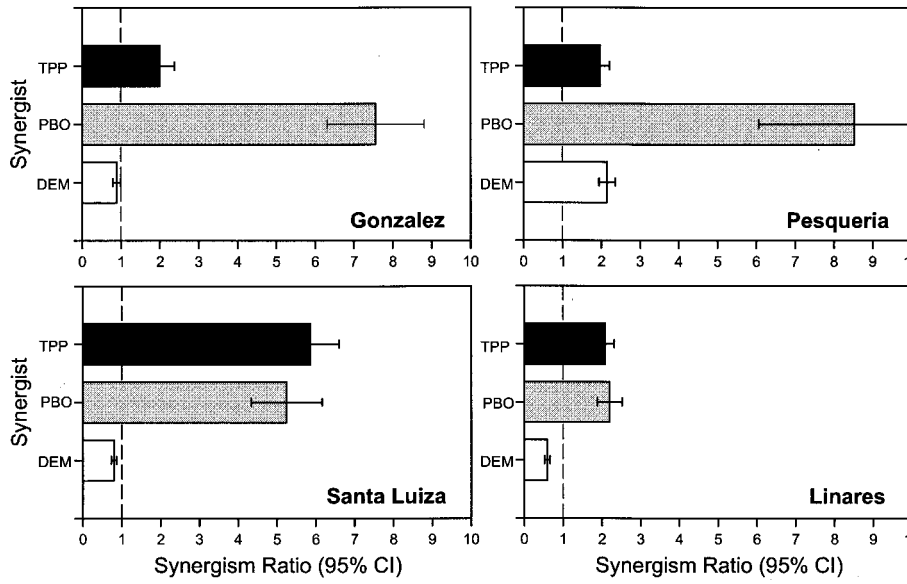

Fig. 1. Effect of synergists on amitraz toxicity in the susceptible reference (Gonzalez) strain and three strains of *B. microplus* with various levels of resistance to amitraz.

Table 3. Resistance to pyrethroid and organophosphate acaricides in the Santa Luiza strain of *B. microplus*

Acaricide/tick strain	*n*	Slope (SE)	χ^2 (df)	LC_{50} (95% CI)	RR (95% CI)
Permethrin					
Gonzalez	1,666	4.98 (0.34)	57.26 (16)	0.0202 (0.0185–0.0226)	1
Santa Luiza	2,275	8.39 (0.90)	54.46 (22)	5.8289 (5.3147–6.7940)	202.89 (188.38–218.52)
Coumaphos					
Gonzalez	2,725	5.00 (0.17)	128.98 (16)	0.0365 (0.0332–0.0403)	1
Santa Luiza	2,479	5.39 (0.32)	111.88 (19)	0.2005 (0.1674–0.2246)	5.50 (5.17–5.85)
Diazinon					
Gonzalez	2,409	3.15 (0.15)	71.48 (19)	0.0185 (0.0158–0.0210)	1
Santa Luiza	2,494	2.93 (0.13)	46.60 (19)	0.0486 (0.0432–0.0543)	2.62 (2.38–2.89)

Resistance to Pyrethroid and OP Acaricides in the Santa Luiza Strain. Table 3 summarizes the bioassay results that compare the susceptibility to permethrin, coumaphos, and diazinon in the Santa Luiza strain with the Gonzalez strain. Compared with the susceptible reference strain, the Santa Luiza strain demonstrated high resistance to permethrin (RR = 202.89) and moderate resistance to coumaphos (RR = 5.50) and diazinon (RR = 2.62).

Discussion

Detection and management of amitraz resistance in *B. microplus* has been a difficult issue due to the lack of a proper bioassay technique that allows clear separation between resistant and susceptible ticks (Kemp et al. 1998). The traditional FAO LPT technique works well in measuring resistance to chlorinated hydrocarbon, organophosphate, and pyrethroid acaricides in *B. microplus* (Miller et al. 1999). However, it is not suitable for measuring amitraz resistance because it produces dose-mortality lines with extremely low slopes (Kemp et al. 1998). The successfully modified LPT by using nylon fabric as substrate has made it possible to measure and compare amitraz susceptibility in different strains of *B. microplus* (Miller et al. 2002). The study reported here is the first application of this modified LPT technique in detecting and measuring amitraz resistance in *B. microplus* populations from Mexico. A modified Shaw's larval immersion test is currently used to measure amitraz resistance in *B. microplus* in Mexico (Soberanes et al. 2002). A study is currently in progress to compare these two bioassay techniques regarding to their sensitivity and repeatability in the detection of amitraz resistance (R.J.M., unpublished data).

Our study of amitraz resistance in *B. microplus* populations from several major cattle-producing areas of Mexico demonstrated low-order (RR = 1.68–4.58) amitraz resistance in 11 of the 15 Mexican strains surveyed. Using the modified Shaw's larval immersion test, Soberanes et al. (2002) reported an RR of 41.9 to amitraz in the San Alfonso strain of *B. microplus* in Mexico. Because different bioassay techniques were used in the two studies, our results may not be directly comparable with theirs. Nevertheless, the confirmation of low-order resistance to amitraz in most *B. microplus* strains surveyed is alarming, particularly given the fact that *B. microplus* has the potential to develop high levels of resistance, as was demonstrated in the Santa Luiza strain (Table 2). The Santa Luiza strain responded to selection quickly, and the resistance ratio was elevated from 13.3 in F_1 to 154 in F_6, after only four generations of selection. Although resistance decreased sharply without selection in the following generations, resistance stabilized at ≈50-fold after resumption of late selection. In Mexico, the level of resistance to amitraz in the San Alfonso strain decreased from 42-fold in F_1 (Soberanes et al. 2002) to 10-fold in F_6 after six generations of laboratory colonization without selection (A.Y.L. and H. Fragoso, unpublished data). Cost of fitness related to amitraz resistance in *B. microplus* may have contributed to the observed decreases in amitraz resistance levels in certain generations of the Santa Luiza strain, as well as in the San Alfonso strain. Further study is needed to determine the possible adverse effects of amitraz resistance on tick feeding and development. Fitness cost associated with pesticide resistance has been reported in many arthropod species (McKenzie 1996). If confirmed to be the case here, fitness cost as a consequence of resistance will certainly have an impact on the development of amitraz resistance in the field.

Results of our synergism bioassays with TPP and PBO clearly demonstrated that both esterases and the mixed function oxidases (MFOs) enhanced amitraz toxicity to *B. microplus* (Fig. 1). However, the contribution of these metabolic enzymes in amitraz resistance is less evident and variable. Although the Linares strain showed a 2.03-fold resistance, the PBO and DEM synergism ratios were smaller than those of the Gonzalez strain, whereas the TPP synergism ratio remained the same. A similar TPP synergism ratio was found in the Pesqueria strain. The only difference between the Pesqueria strain and the Gonzalez strain is that the DEM significantly synergized amitraz toxicity in the Pesqueria strain. It is likely that glutathione *S*-transferases may have played a role in the low-order amitraz resistance observed in the Pesqueria strain. An earlier study on resistance to OPs indicated that there was a significant correlation between the DEM synergism ratio and diazinon resistance in the Mexican strains of *B. microplus* (Li et al. 2003). The Pesqueria strain is highly resistant to diazinon (Li et al., unpublished data). It is possible that the activity of glutathione *S*-transferases was elevated in the diazinon-resistant Pesqueria strain, and those enzymes may also have contributed to the observed resistance to amitraz

in this strain. In the Santa Luiza strain, the TPP synergism ratio was significantly higher than in the Gonzalez strain, whereas the PBO synergism ratio was significantly lower and DEM had no effect.

Our synergism bioassays showed that PBO synergized amitraz toxicity in both resistant and susceptible strains. An earlier study on amitraz metabolism in *B. microplus* larvae also demonstrated that PBO had a threefold synergistic effect on amitraz toxicity but had only a slight effect on amitraz metabolism (Schuntner and Thompson 1978). Because formamidine acaricides, particularly amitraz, are MFO inhibitors themselves, the apparent synergism of amitraz by PBO, also an inhibitor of MFOs, could be the result of a simple additive effect (Schuntner and Thompson 1978).

Among the Mexican strains of *B. microplus* colonized and studied at the CFTRL, the San Felipe and Coatzacoalcos strains were resistant to pyrethroids (Miller et al. 1999), and the Tuxpan, Linares, Pesqueria, Caporal, and San Roman strains are resistant to OPs (Li et al. 2003). It is interesting to note that all OP-resistant strains, except the Tuxpan strain, demonstrated significant levels of amitraz resistance. Additionally, our initial amitraz bioassay in 1999 did not detect any amitraz resistance in the San Roman strain (data not shown). This strain has been selected with coumaphos for 3 yr before the latest amitraz bioassay was performed in 2002. Although the synergist bioassay results from this study did not suggest an involvement of MFOs in amitraz resistance, our bioassay data on those OP-resistant strains indicate a link between amitraz and OP resistance. Our recent study on coumaphos resistance in the laboratory maintained *B. microplus* strains (number 1 through 8) indicated an enhancement of MFO activity in the same OP-resistant strains (Li et al. 2003), which may have caused the apparent cross-resistance between amitraz and coumaphos resistance in those strains. In contrast, the San Felipe strain had a 6.5-fold resistance to amitraz when first tested in 1999 (data not shown), and the level of amitraz resistance declined to 1.79-fold in 2002. The San Felipe strain has been challenged with permethrin during this period of time.

Although a higher TPP synergism ratio may suggest a possible contribution of hydrolyzing esterases in amitraz resistance in the Santa Luiza strain, it certainly does not explain the high amitraz resistance level observed in this strain. The main target of amitraz action is believed to be the octopamine receptor in the central nervous system of insect species (Evans and Gee 1980, Dudai et al. 1987). The high level of amitraz resistance observed in the Santa Luiza strain strongly suggests that reduced sensitivity to amitraz binding at the octopamine receptor in the nervous system might be the major mechanism of resistance to amitraz in this strain of *B. microplus.* Further study on interaction of amitraz and octopamine receptor is needed to elucidate the mechanism of amitraz resistance, as well as to develop biochemical and molecular diagnostic tools for rapid detection of amitraz resistance in *B. microplus.*

It was reported that the amitraz-resistant San Alfonso strain from Mexico was also highly resistant to pyrethroids and moderately resistant to OPs (Soberanes et al. 2002). Our study on the Santa Luiza strain, which originated in Brazil, demonstrated a similar resistance profile (Table 3). In Australia, the Ultimo strain of *B. microplus* was found to be resistant to amitraz and all available pyrethroids (Kunz and Kemp 1994). Multiple resistance to all three major classes of acaricides discovered in *B. microplus* from different regions of the world has serious implications to future tick control strategies. Measures must be taken to slow resistance development and the spreading of amitraz resistance in Mexico. Existence of widespread low-order resistance to amitraz in Mexican populations of *B. microplus* also has serious implications on the strategies the USDA may adopt in the future to prevent *B. microplus* from reentering the United States from Mexico through cattle importation. The low-order resistance detected in most of *B. microplus* strains from Mexico also suggests that the frequency of gene(s) that confer amitraz resistance was low and may exist as heterozygotes in most field populations. We are currently conducting a genetic study to investigate the inheritance of amitraz resistance in the Santa Luiza strain of *B. microplus.* Knowledge of the inheritance mechanisms and relative susceptibility of different genotypes, as well as their frequency in field populations of *B. microplus*, would help to predict the evolution of amitraz resistance and to develop effective control strategies aimed at reducing or slowing amitraz resistance development.

Acknowledgments

We thank Dave Krska and Melinda May for excellent technical assistance; Horacio Bazan, Adolfo Pena, and Homero Vasquez for maintaining tick strains and handling animals; and Mario Villarino for collecting from Mexico some of the tick strains used in this study. We also thank Andrew C. Chen, Patti J. Elzen, and Timothy J. Lysyk for reviews of an earlier draft of this manuscript, and two anonymous reviewers for comments on the submitted version of this manuscript.

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Received for publication 27 May 2003; accepted 1 October 2003.